CHEMICAL ANATOMY OF THE PERICARDIAL AND PERIVISCERAL FLUIDS OF THE STINGRAY, DASYATIS AMERICANA[1]

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The significance of the observation by Staedeler and Frerichs (1858) of "colossale Quantitaten" of urea in the blood and tissues of some skates and a shark remained obscure for four decades. In 1897 Bottazzi reported that the freezing point depressions of elasmobranch blood and of sea water were approximately equal, that is, they had approximately the same total solute particle, or osmolal, concentrations. But whereas sodium and chloride make up the bulk of solute particles in sea water, Rodier (1899) observed that urea accounted for about one-third of the solute particles of elsamobranch blood. Rodier also noted that the freezing point depression of the fish blood was generally slightly greater than that of the animal's sea-water environment, an observation which has been repeated often (Fredericq, 1904; Garrey, 1905; Bottazzi, 1906; Scott, 1913; Smith, 1936). In shallow waters, such as those generally used for collection of experimental animals, the environmental solute concentration may fluctuate depending upon (among other factors) the season, height of tide, location, depth of water, and time of day. Thus, one could expect that the freezing point depression (total osmolality) of elasmobranch blood would vary depending upon the environment (see Table I). Experiments by Scott (1913) demonstrated that the urea of the blood was not the only solute to be affected by an environmental concentration change; sharks immersed in hypo-osmotic environments lost chloride, probably *via* the gills. Smith and Smith (1931) reported that the blood of fresh-water elasmobranchs contains decidedly less urea and chloride than that of marine elasmobranchs. More recently, Hartman *et al.* (1941) found that injection of urea into the vascular system of *Raja erinacea* caused a decrease in the blood's sodium concentration. They also observed that during inanition blood urea fell and sodium became elevated. Apparently, as Smith had observed in 1936, there is a reciprocal relationship between the concentrations of salt and urea.

The first comprehensive survey of the inorganic solutes of elasmobranch blood was made by Homer Smith (1929). His data plus other data from the literature are summarized in Tables II and III. Most notable are the relatively high and somewhat variable levels of sodium and chloride in the blood. The apparently variable levels of blood calcium, magnesium and potassium may actually reflect difficulties of analysis rather than extreme variation within the group.

Many investigators have measured the blood urea nitrogen (BUN) of elasmobranchs. As one could expect from what has been said above, the values range widely: from 750 mg.% (Scott, 1913) to 1100 mg.% (Smith, 1929) in blood

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TABLE I

Published mean freezing-point values for plasma or serum samples from various elasmobranchs. Synonymies have been checked in Bigelow and Schroeder (1948, 1953). In some cases the freezing point has been calculated from data expressed as milli Osmoles/Kg.

Group	Species	Δ (° C.)*	Reference
Selachians			
	Mustelus canis	−1.79	Doolittle *et al.* (1960)
	Mustelus canis	−1.87	Scott (1913)
	Scyliorhinus caniculus	−2.06	Para (1936)
	S. stellaris	−2.3−−2.42	Bottazzi (1897)
	Scyllium sp.	−2.20	Duval (1925)
	Squalus acanthias	−1.80	Dakin (1908b)
		−1.81	Maren (1962)
		−1.83	Stone and Dewell (1962)
		−1.84	Scott (1913)
		−1.87	Rodnan *et al.* (1962)
		−1.89	Burger and Hess (1959)
		−1.90	Denis (1913)
		−2.04	Macallum (1926)
Batoids			
	Dasyatis americana	−1.64	Wynn and Bernard (1964)
	Raja sp.	−1.88	Garrey (1905)
	R. batis	−1.82	Dakin (1908a)
	R. clavata	−2.04	Para (1936)
	R. erinacea	−1.80	Chaisson and Friedman (1933–34)
	R. laevis	−1.78	Maren *et al.* (1963)
	R. ocellata	−1.73	Maren *et al.* (1963)
	R. radiata	−1.51	Dakin (1908a)
	*R. undulata***	−2.07	Para (1936)
	Trygon sp.	−2.38	Bottazzi (1897)
		−2.44	Mosso (1890)

* Data presented by Bottazzi (1897) indicate that the colloidal solute particles present in the plasma are responsible for a very minor portion of the freezing point depression effect.

** Probably a guitarfish, *Rhinobatos undulatus*.

samples from *Mustelus canis,* and from 870 mg.% to 1320 mg.% in samples from different species of *Raja* (Smith, 1929). The highest BUN level was 1380 mg.% reported for *Scyllium catulus* (von Schroeder, 1890). The blood's non-protein nitrogen (NPN) level has been measured by Denis (1913, 1922), Smith (1929), and Cohen *et al.* (1958). In *Carcharhinus obscurus, Carcharias littoralis* (= *C. taurus*), *Mustelus canis* and *Squalus acanthias* the NPN level ranged from 1000 to 1180 mg.%; all but about 130 to 190 mg.% was accounted for as urea. Trimethylamine oxide and betaine are probably responsible for most of the excess (Smith, 1936; Cohen *et al.,* 1958). Ammonia is present in very small amounts in elasmobranch blood. Smith (1929) was unable to detect any ammonia in the blood of *M. canis* or of *Raja diaphenes* (= *R. ocellata*) and only a trace in *C. littoralis.* Denis (1922) reported blood ammonia levels of 1.6 mg.% for *M. canis* and 1.7 mg.% for *C. obscurus.* The nutritional states of the animals could account for the two-fold differences (28 and 56 mg.%) in amino acid nitrogen levels reported for *M. canis* by Denis (1922) and Doolittle *et al.* (1960), respectively.

C. obscurus had 31 mg.% and *C. taurus* had 39 mg.% according to Denis (1922). The total protein content of elasmobranch plasma is also highly variable. Of 13 species which have been examined the lowest value was 1250 mg.% for the plasma of *Rhinobatos productus* (Urist, 1961). Reported values generally are in the range of 2800 to 3200 mg.% (Dill *et al.,* 1932; Para, 1936; Cohen *et al.,* 1958; Doolittle *et al.,* 1960; and Urist, 1961).

The relationships of the vascular compartment to the external environment, and the osmotic advantages of urea retention and elevated blood salt concentrations of elasmobranchs have been summarized by Smith (1936). But the rela-

TABLE II

Average concentrations of some inorganic solutes in plasma of various elasmobranchs

Group	Species	Solute (mEq./L.)					Reference
		Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-*	
Selachians	*Aprionodon isodon*	238	7.0	—	—	252	Sulya *et al.* (1960)
	Carcharhinus limbatus	258	10.0	—	—	241	Sulya *et al.* (1960)
	Carcharias taurus	268	9.2	9.2	14.1	298	Macallum (1926)
	*Mustelus canis***	288	8.0	5.0	3.0	270	Doolittle *et al.* (1960)
	Scyliorhinus caniculus	303	—	8.5	—	303	Para (1936)
	Squalus acanthias	258	6.9	8.0	12.5	280	Macallum (1926)
	Squalus acanthias	286	5.7	5.2	—	246	Burger and Hess (1959)
	Squalus acanthias	263	4.1	6.6	—	249	Rodnan *et al.* (1962)
	Squalus acanthias	—	6.6	—	—	251	Hogben *et al.* (1960)
	Squalus acanthias	255	6.6	—	—	239	Maren (1962)
	Squalus acanthias	223	3.3	—	—	241	Robin *et al.* (1964)
	Sphyrna tiburo	289	12.5	—	—	245	Sulya *et al.* (1960)
Batoids	*Platyrhinoidis triseriata*	234	11.4	10.5	9.1	208	Urist (1961)
	Raja clavata	285	4.0	—	—	240	Enger (1964)
	Raja clavata	288	—	7.0	—	287	Para (1936)
	Raja clavata	289	4.0	—	—	311	Murray and Potts (1961)
	R. erinacea	260	3.9	—	—	253	Maren *et al.* (1963)
	R. laevis	182	4.2	—	—	220	Maren *et al.* (1963)
	R. ocellata	285	3.5	—	—	255	Maren *et al.* (1963)

* Scott (1913) reported 246 mEq. of Cl^- per liter for *M. canis* and Chaisson and Friedman (1933–34) a 260 mEq. of Cl^- per liter for *R. erinacea*.

** Compare with data presented in Table III.

tionships of the vascular compartment to other fluid-containing cavities within the elasmobranch body are not as well-known or understood. The aqueous humor has received attention (Kisch, 1930; Doolittle *et al.,* 1960; Stone and Dewell, 1962; Maren, 1962). So have the cranial cavity fluid (Hogben *et al.,* 1960; Murray and Potts, 1961; Maren, 1962) and the fluids of the labyrinth (Kisch, 1930; Murray and Potts, 1961). Pericardial fluid practically fills the rather rigid pericardial cavity and could influence cardiac dynamics. But observations upon the fluid are not numerous and hardly systematized. The same could be said of the rather voluminous perivisceral fluid. For a summary of available data, see Table III.

Our objective was to measure the osmotic relations and the major solute com-

TABLE III

Comparison of average concentrations of some components of the blood, plasma or serum (S), pericardial fluid (PC) and perivisceral fluid (PV) of some sharks and skates. Numbers in boldface represent reported significant differences from plasma values.*

Species	Fluids	pH	Inorganic components (mEq./L.)						Reference
			Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	Urea–N mg.%	
Carcharias taurus	S	7.4	267	5.5	11.0	4.6	235	1060	Smith (1929)
	PV	**5.8**	276	8.9	10.2	**42.8**	306	1000	
	PC	**5.4**	290	**9.0**	**5.7**	5.4	—	—	
Squalus acanthias	S	7.5	263	4.1	6.6	3.1	249	1040	Rodnan *et al.* (1962)
	PV	**5.7**	**296**	4.4	**4.3**	**7.3**	**328**	1210	
Raja erinacea	S		254	8	12	5	255	960	Hartman *et al.* (1941)
	PV		(201–463)	10	(3–18)	42	300	—	
R. laevis	S	7.4	255	4.9	7.5	5.6	241	1270	Smith (1929)
	PV	**5.8**	284	5.8	5.0	**35.8**	309	1280	
	PC	**6.4**	335	**20.3**	**1.6**	5.0	370	**1010**	
R. ocellata	S	7.3	237	6.8	10.2	7.0	227	1050	Smith (1929)
	PV	**5.8**	156	6.2	6.4	—	188	**715**	

* Kisch (1930) reports all three fluids had about the same amount of urea in *Torpedo ocellata* (about 800 mg.%) and the plasma and pericardial fluids of *Trygon violacea* had equivalent amounts (1090 mg.%).

ponents of stingray perivisceral and pericardial fluids and relate these to the plasma constituents and to the environment. We have made a preliminary report on our 1963 collections (Wynn and Bernard, 1964). It should be recalled that in elasmobranchs in general the coelomic cavity is potentially in continuity with the environment *via* abdominal pores, and that the pericardial cavity is potentially in continuity with the peritoneal cavity *via* pericardioperitoneal canals in the transverse septum.

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MATERIALS AND METHODS

Animals were captured during the daylight hours at low water in the shallows off Sea Horse Key, Florida, or neighboring small islands of the Cedar Keys (29°8′N.; 83°2′E.). Periods of collection were: March 15–19, June 3–14, and July 22–30, 1963 and June 8–12, 1964. Stingrays were speared through a "wing" (pectoral fin) and then quickly hoisted aboard the skiff. Some rays had been pursued before capture. In 1963, 25 specimens of *Dasyatis americana* were cap-

tured; of the 11 specimens captured in 1964, four were of another species, *D. say*. In 1963 only one male was collected; 9 of the females were pregnant. In 1964, two males, both *D. say,* were collected; three females, all *D. americana,* were pregnant. The average width of all the *D. americana* specimens was 71 cm. (range: 41–92 cm.).

After inverting the animal the ventral surface was blotted dry. A U-shaped incision beginning and terminating at the coracoid bar was made. After reflecting the skin and hypobranchial musculature posteriorly, the pericardial sac was exposed and incised. A sterile, chemically clean eyedropper was used to collect the sample which was immediately placed in a sterile, previously unused, chemically clean, rubber-stoppered, 10-ml. tube (B-D Vacutainer). Because of the relatively large volume of fluid, sometimes two tubes had to be utilized. The perivisceral fluid was collected in the same way through a midline abdominal incision. Neither incision resulted in noticeable hemorrhage. While blood was being collected, the pH of aliquots of the pericardial and perivisceral fluids was measured, first with a Photovolt Model 180 pH meter and then with Hydrion narrow range pH papers (Micro Essential Laboratory, Brooklyn, New York). For blood collection an 18-gauge needle attached to a 10-ml. syringe was inserted into one of the posterior cardinal sinuses and the blood gently aspirated. Later we found that blood was more conveniently obtained by cardiac puncture. The blood sample was carefully transferred to a 15-ml. centrifuge tube. After 5 minutes centrifugation in a hand-powered centrifuge, the serum was separated, and its pH measured. The serum was then placed in a Vacutainer tube. Upon filling, all tubes were partially evacuated and placed in a dry-ice cabinet for quick freezing of the samples. The samples were kept frozen until other determinations were made. Total time elapsing between capture and fluid collection seldom exceeded 15 minutes. When fluid collection was completed the animals always had active cardiac movements and generally had active respiratory movements.

Total osmolality was determined cryoscopically with a Fiske Model H osmometer. Total protein was estimated by the method of Lowry *et al.* (1951); urea-N and NH_3-N, by a modification of the microdiffusion method of Conway (Hawk *et al.*, 1954); Na^+ and K^+, by flame spectrophotometry; Cl^-, by the method of Schales and Schales (1941); Ca^{++}, by the method of Bachra *et al.* (1958); and Mg^{++}, by the method of Andreasen (1957). Amino acid concentrations were determined by chromatographic separation on a Spinco Amino Acid Analyzer, Model 120B.

Results

The results of the fluid analyses are summarized in Tables IV and V.

In comparison to the slightly acid serum, the pericardial and perivisceral fluids are considerably more acid. We also observed that the acidity diminished during the first 90 minutes after collection. As others have observed in other elasmobranch species (Table I), the stingray's blood is hyperosmotic to the seawater environment which, in the area where our specimens were collected, is itself highly variable osmotically. Our 1964 data indicate that the fluid contents of the two serous cavities have significantly fewer solute particles than the vascular

TABLE IV

Composition of D. americana and D. say body fluids. In the case of D. americana the data are expressed as mean values ± standard deviation and the number of observations is in parentheses below. Because only a few data were obtained for D. say (all collected in 1964), only the means are tabulated. Abbreviations as in Table III.

	Fluid	pH	mOsm./Kg.	Inorganic solutes (mEq./L.)					Organic solutes (mg.%)		
				Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	urea-N	NH_3-N	Total protein
D. americana (1963 data)	S	6.3±0.4 (2)	883±69 (18)	238±25 (20)	17.0±4.3 (18)	8.2±1.5 (13)	3.2±0.9 (8)	238±23 (10)	1064±158 (18)	4.4±2.5 (17)	4120±1520 (18)
	PV	5.3±0.1 (5)	849±91 (13)	239±22 (16)	14.0±2.0 (13)	3.1±1.2 (16)	0.7±0.2 (18)	260±27 (15)	1126±217 (13)	50±17 (13)	137±48 (11)
	PC	5.5±0.3 (8)	861±88 (19)	246±40 (25)	10.0±1.8 (21)	1.1±0.3 (20)	1.6±0.6 (14)	287±30 (23)	1020±204 (18)	36±12 (18)	41±21 (19)
D. americana (1964 data)	S	6.9±0.0 (4)	864±30 (6)	251±23 (6)	18.8±1.5 (6)	23.2±0.9 (6)	3.8±0.9 (6)	256±11 (6)	983±70 (6)	2.3±0.8 (6)	3550±700 (5)
	PV	5.4±0.1 (6)	827±23 (6)	255±12 (6)	20.4±1.1 (5)	5.1±1.1 (6)	0.6±0.1 (6)	310±31 (6)	1021±51 (6)	31±8.4 (6)	111±69 (6)
	PC	5.4±0.1 (6)	828±22 (6)	262±13 (6)	11.9±0.3 (6)	1.8±0.6 (6)	1.3±0.4 (6)	308±31 (6)	951±52 (6)	23±2.4 (6)	31±9 (6)
D. say	S	6.0	840	256	20.6	19.5	3.1	262	1069	2	2600
	PV	5.5	813	236	20.5	7.7	3.0	289	998	44	153
	PC	5.4	810	258	11.7	3.7	1.6	295	992	37	40
Sea water—1963		8.2±0.0 (2)	836±18 (3)	405±25 (3)	14.0±1.0 (3)	37.1 (1)	—	424±0.0 (2)	None detectable		
Sea water—1964		8.2±0.0 (2)	709±22 (6)	346±7.2 (6)	21.4±1.5 (6)	40.1±2.5 (6)	59±6.4 (6)	399±11 (6)	None detectable		

TABLE V

Probability values derived from comparison of body fluids of D. americana. A dash indicates no significant difference, i.e., P. >0.05. Abbreviations as in Table III.

Fluids	Year	pH	Osmolality	Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	Urea	NH_3-N	Total protein
S:PC	1963	<0.05	—	—	<0.01	<0.01	<0.01	<0.01	—	<0.01	<0.01
	1964		<0.05	—	<0.01	<0.01	<0.01	<0.05	—	<0.01	<0.01
S:PV	1963	<0.05	—	—	<0.05	<0.01	<0.01	<0.05	—	<0.01	<0.01
	1964		<0.05	—	—	<0.01	<0.01	<0.05	—	<0.01	<0.01
PC:PV	1963	—	—	—	<0.01	<0.01	<0.01	<0.05	—	<0.05	<0.01
	1964	—	—	—	<0.01	<0.05	<0.05	—	<0.05	—	<0.05

compartment. Although the 1963 data were similar, the variability was greater and the differences were not significant.

Within the stingray, both Na^+ and Cl^- concentrations were less than environmental concentrations and apparently were independent of rather large shifts in the latter (*cf.* 1963 and 1964 sea-water data). There were no significant differences of sodium concentration between the three compartments. Chloride, on the other hand, was significantly elevated in the two serous cavities. The pericardial fluid contained significantly less potassium than either the serum or perivisceral fluid. The bivalent cations, magnesium and calcium, were present in significantly smaller amounts in the two serous fluids. The pericardial fluid contained significantly less calcium and significantly more magnesium than the perivisceral fluid.

Like sodium ions, urea molecules were in equilibrium between the blood and the two serous cavities; ammonia, on the other hand, was much more concentrated in the perivisceral fluid and only slightly less so in the pericardial fluid. Corresponding to the elevated Cl^-, there was very little protein in the two serous fluids.

Fluids obtained from *D. say* were (with the exception of Mg^{++} concentration in the perivisceral fluid) chemically similar to those from *D. americana.*

Eleven amino acids were detected in a sample of pericardial fluid from *D. americana.* Their concentrations (in micromoles per cent) were: aspartic acid (0.71), threonine (0.52), serine (2.77), asparagine plus glutamine (0.76), glycine (3.00), alanine (1.16), isoleucine (0.40), leucine (0.97), ornithine (1.09), and taurine (3.09). In addition there was noted a "possible trace" of arginine, and two unknown ninhydrin-positive compounds present in relatively large amounts (3.93 and 2.77 micromoles per cent).

DISCUSSION

The relative acidity of the pericardial and perivisceral fluids of several species had been observed by Smith (1929). Rodnan *et al.* (1962) made the same observation upon the perivisceral fluid of *Squalus* (see Table III). Our generally

lower values might reflect species differences, or collection techniques which provided fluid samples under optimum field conditions.

As noted above, elasmobranch blood osmolality is highly variable and related to the environmental osmolality. Our observations would indicate that the relationship may not be too rigorously regulated. Osmolar differentials exist between the three fluid compartments, with the blood hyperosmotic to the serous fluids.

Since there have been no comparable studies on other stingray species, comparisons of *D. americana* or *D. say* solute concentration data with data reported for remotely-related species would have little value. In general, our data are not notably different from those reported by others. There are two noteworthy exceptions: Rodnan *et al.* (1962) reported significantly more sodium in the perivisceral fluid of *Squalus.* We found no difference. Secondly, Campbell (1961), using a different analytical technique, reported that the plasma of the closely related species, *D. centrura,* contains up to 32 micromoles per cent of arginine. This is about ten times the concentration of any amino acid the amino acid analyzer was able to detect. In fact, we found only a "trace" of arginine. Arginine present in our samples originally might have been lost through long storage.

The data, as well as those reported elsewhere in the literature, clearly indicate that artificial physiological bathing fluids simulating plasma may not contain optimal ion concentrations for all elasmobranch viscera. In studies of elasmobranch heart physiology, for example, not only would one have to consider the almost rigid musculo-skeletal ensheathment of the pericardium (Lyon, 1926; Dohrn and Rein, 1949–50) and the apparently normal, and perhaps physiologically necessary, cardiac tamponade but also the factors of high H^+ and low K^+, Mg^{++} and Ca^{++} concentrations in the synthesis of any pericardial fluid substitute.

That the abdominal pores in these species of stingray probably do not permit entrance of sea water can be inferred from the gross ionic dissimilarities between sea water and perivisceral fluid. (Of course, special ion "pumps" might be present.) Similarly, mechanical exchange of fluids through the pericardioperitoneal canals must be limited. Because of the tamponade and respiratory movements, passage from the pericardial cavity to the abdominal cavity is more likely than flow in the opposite direction.

Summary

1. The pH, osmolarity, Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, urea, NH_3 and total protein content of the serum (S), pericardial fluid (PC) and perivisceral fluid (PV) of the stingray, *Dasyatis americana,* were estimated and compared to environmental data.

2. The PC and PV were found to be more acid and osmotically less concentrated than S. Although there were no significant differences in either Na^+ or urea concentrations in the three body fluids, K^+ was significantly reduced in PC. Ca^{++} and Mg^{++} were significantly less concentrated in PC and PV. Both PV and PC had elevated Cl^- and almost no protein; both contained significantly elevated NH_3 concentration. It follows that recipes for synthetic bathing fluids for elasmobranch viscera should not be based upon data obtained from plasma or serum analyses.

3. A few specimens of *D. say* were collected. The data were similar.

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